SHORT INSTRUCTIONS FOR OPERATING SD2 AT CIAN

Version 2, October 2009

Before starting

To work with SD2, you need to get trained. Contact Judith Lacoste (judith.lacoste@mcgill.ca) or Guillaume Lesage (guillaume.lesage@mcgill.ca, 514-398-5414) to organize a training session.

General workflow

1. Turn on microscope, components as needed (see sections 1, 2), log in logbook
2. Start up computer, log into Volocity, calibrate stage (see section 2)
3. Visually inspect objectives; clean as needed (section 2.2)
4. Use test slide to adjust Köhler illumination in bright field (section 2.1)
5. Focus and position experimental sample in transmitted light (section 3.1)
6. Use acquisition software to acquire images, stacks, time lapses (see section 3.2)
7. When done, send light to eyepiece, clean microscope workstation as needed
8. Turn off microscope (see section 4), log time in logbook
9. Move data off computer (see section 5)

General reminders for SD2

- Save the camera: move light to eyepiece when not acquiring data; avoid overexposure by checking intensity levels
- Optical cables are fragile, don't touch or put things on them
- Avoid bumping into equipment, leaning on air table
- Move data off microscope computer – if storage space runs out during an acquisition, all data is lost for that library!
- Limit or omit use of non-confocal (wide field) fluorescence; kills sample and signal
- Volocity time = pay time; log off when not needed

Useful abbreviations and terms

SD = spinning disk

BF = bright field

TL = transmitted light

IL = incident light, i.e. fluorescent light

FA = field aperture, also AP = aperture diaphragm

FD = field diaphragm

WFF = wide field fluorescence

DIC = Differential Interference Contrast, a.k.a. Nomarski Interference Contrast

LMM = Laser Merge Module, merges the light paths of all SD2 lasers into one optical fibre cable

Volocity = acquisition and analysis software from Improvision (Perkin-Elmer)

Light sources on SD2: halogen lamp (for TL), *Exfo* (for WFF), lasers (for SD confocal)

More information

The *Leica* DMI6000B operating manual is available at http://biology.mcgill.ca/CIAN/microscopy.htm

The *Volocity* user manual can be found in the 'Shared' folder on the CIANsan server.

1 - Equipment Setup

Fig 1: Basic components of the SD2 microsope; brand names in italics.

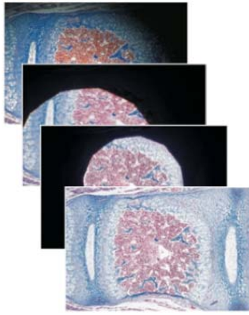
1. Laser shutter controller (leave ON)
2. Laser ignition (4 boxes, “xxx nm” on label)
3. *Ludl* emission filter controller, halogen lamp shutter
4. Camera, Faraday cage, “grounded”
5. Emission filter wheel
6. Confocal scanning head
7. Fiber optical cable
8. *Leica* DMI 6000B inverted microscope
9. Halogen lamp, bright field light source
10. Joystick for XYZ stage control
11. Air table
12. *APC* UPS (uninterruptable power supply)
13. Computer
14. Controller for environmental control chamber
15. *Leica* electronic box, microscope controller
16. *Exfo* metal halide light source for widefield fluorescence

2 - Starting up SD2

Note: Diode lasers require only minimal warm up, but adhere to **15min/15min** rule: leave on at least 15min before shutting off, leave off at least 15min before restarting; 1h/1h rule applies for *Exfo* lamp

1. Log on the **logbook**, indicate your name, the date, time in, laser(s) used
2. Take the **dust cover** off the microscope, put on wardrobe hooks by the door, not the floor
3. Remove any **stage inserts**, CO_2 chamber, objective warmer to make sure that the stage does not hit anything when moving
4. **Turn on** microscope system by pressing the top (‘test’) button on the *APC* UPS (Fig 1-12)
5. Turn on **computer** (Fig 1-13), log in to your account, start Volocity, calibrate stage (‘Stage : Calibrate Stage’)
6. Optional, as needed: turn on lasers (Fig 1-2), *Exfo* widefield fluorescent light source (Fig 1-16), environmental control (Fig 1-14), CO_2 tank
7. Visually inspect **objectives**, clean as needed (see section 2.2)

2.1 - Adjusting Koehler Illumination (more detailed instructions are in development)



1. Insert your test slide, adjust light intensity for eye comfort, focus on specimen in bright field using 10x objective
2. Close FD to see edge, focus it by adjusting the condenser height, center if needed, open FD just enough to illuminate field of view (see images on the left)
3. adjust FA if needed
4. In case of poor images, reclean objective (see section 2.2), repeat procedure
5. Repeat for every objective to be used

2.2 - Cleaning objectives

Clean objectives before and after use as follows:

- Take a Kimwipe and fold it in three into a long rectangle.
- Wipe the lens gently by holding either end of the Kimwipe, dragging it gently across the objective lens three times. Use a fresh area of the tissue each time.
- Repeat as needed to remove excess of immersion oil.

Use a test slide to evaluate the optic of the objective, and if needed, clean the objective more thoroughly as follow:

- Fold as above, put a drop or two of lens cleaner (the blue fluid) on the Kimwipe.
- Wipe the lens gently by holding either end of the wet lens tissue dragging it gently across the objective lens three times. Use a fresh area of the tissue each time.
- Repeat with water, then with ethanol.
- Give the objective lens a final wipe or two with a dry Kimwipe to remove any excess fluid.

NEVER wipe the lens in a circular pattern.
NEVER apply any pressure directly to the lens.

3 - Operating SD2 - *Report any problems in the logbook and to Judith or Guillaume.*

3.1 - Manual procedure

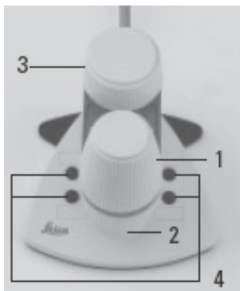


Fig 2, Joystick for stage control
1: travel in Y
2: travel in X
3: adjust focus (travel in Z)
4right: Z fine/coarse
4left: XY fine/coarse

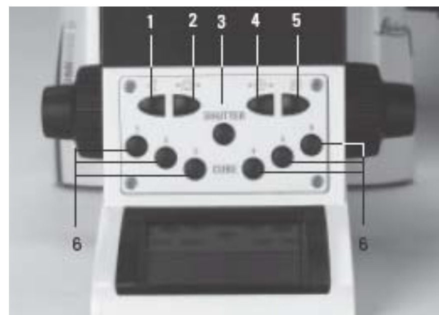


Fig 3, Microscope front panel
1: 100% to eyepiece
2: 100% to camera
3: WFF shutter
4 and 5: unused
6: Selecting filter cubes

Wide field fluorescence

NOTE: the regular fluorescence and spinning disk CANNOT work simultaneously.

1. Turn on the *Exfo* light source (Fig 1-16) when needed. This light source has no refractory period.
2. Switch to incident light fluorescence excitation using the TL/IL button (left panel), or the 'fluo' button (right panel)
3. Select the filter cubes using the filter selector on the front panel (Fig 3-6)
4. The *Exfo* lamp has to be on for at least one hour before it is turned off.

3.2 - Using *Volocity* for microscope control and image acquisition

Log in to '*Volocity 3DM* acquisition' using your License server login and password. Other license types: 'Vogel Volocity' (all), 'Restore class' (deconvolution), 'Visual class' (3D rendering), 'Presentation' (free, limited edition, can be run without license).
Create a new library. Select **<Show Video Preview>** from the Window menu.

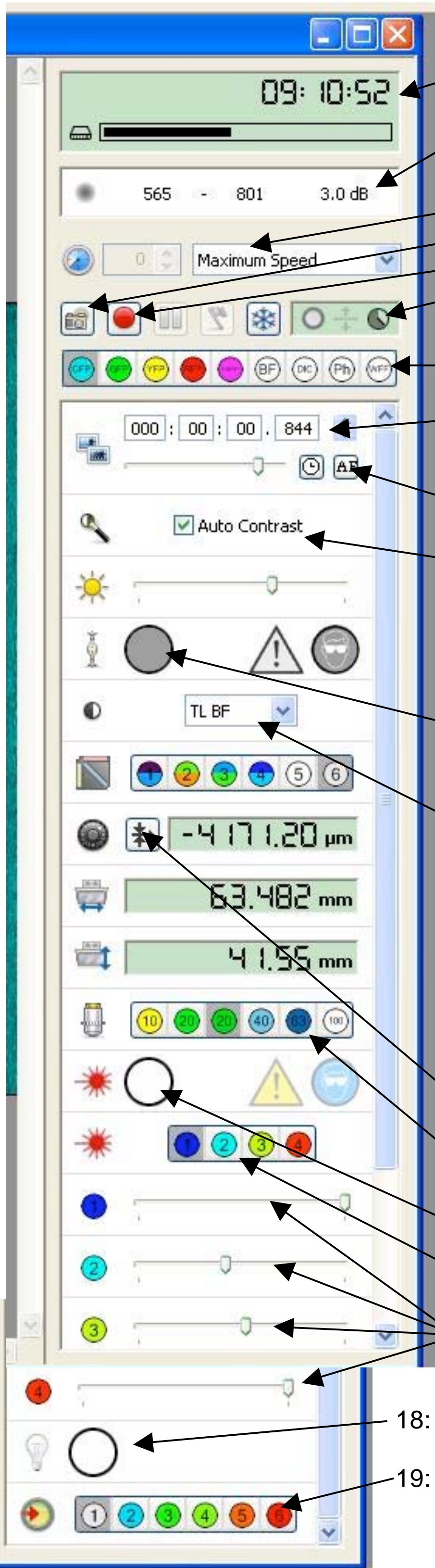


Fig 4: Device controls in Video Preview window

1: Time or elapsed time, hard disk storage space

2: Pixel intensity min. and max. – can toggle to different views of signal intensity, e.g. signal/time graph for bleaching evaluation

3: Acquisition rate/frequency – scroll to select

4: Shoot – click to shoot a single screen shot

5: Acquire – start recording with current settings

6: Acquisition setup – double-click to see menu (see section 3.2.2)

7: Light path manager
- nine light paths are preconfigured, specific configurations can be made upon request
- CFP confocal
- GFP confocal
- YFP confocal
- RFP confocal
- FRFP confocal
- BF, bright field
- DIC
- Phase contrast
- WFF, wide field fluorescence; defaults to GFP settings, others have to be selected manually

8: Exposure time

9: Auto exposure (use with caution)

10: Auto contrast (use recommended)

11: Wide field fluorescence shutter - click to close/open, redundant to shutter button on microscope (Fig 3-3)

12: Contrast modes – for confocal operation, leave on TL BF
- TL BF (transmitted light, brightfield)
- TL PH (Phase contrast, 10x and 20x [pos.3])
- TL DIC (Differential Interference Contrast, 20x [pos.2], 40x, 63x, and 100x)
- TL POL (Polarizer light)
- FLUO (regular fluorescence)
- FLUO/PH (mixed regular fluorescence and Phase)
- FLUO/DIC (mixed regular fluorescence and DIC)

13: Focus control – use for Z-stack setup (see section 3.2.1)

14: Objective indicator – turn objectives manually only!

15: Confocal fluorescence shutter (LMM5) – click to close/open
Make sure to close shutter prior to logging out of Volocity

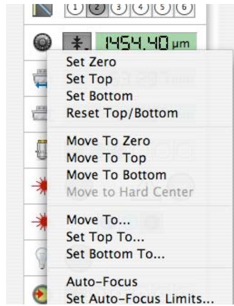
16: Laser line indicator (LMM5) – visualize laser(s) in use

17: Laser intensity controls (neutral density filters) – note that the controls are only linear between 60% and 80%

18: shutter for halogen lamp = bright field light source (*Ludl* controller)

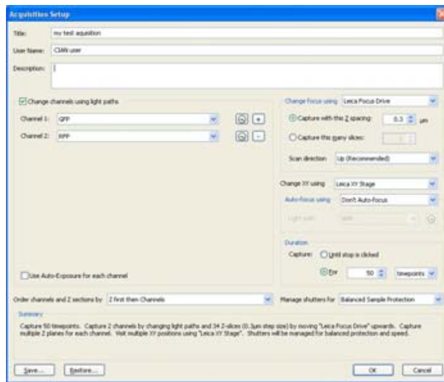
19: Confocal emission filter indicator – should match light path

3.2.1. Z-stack setup for relative stack size



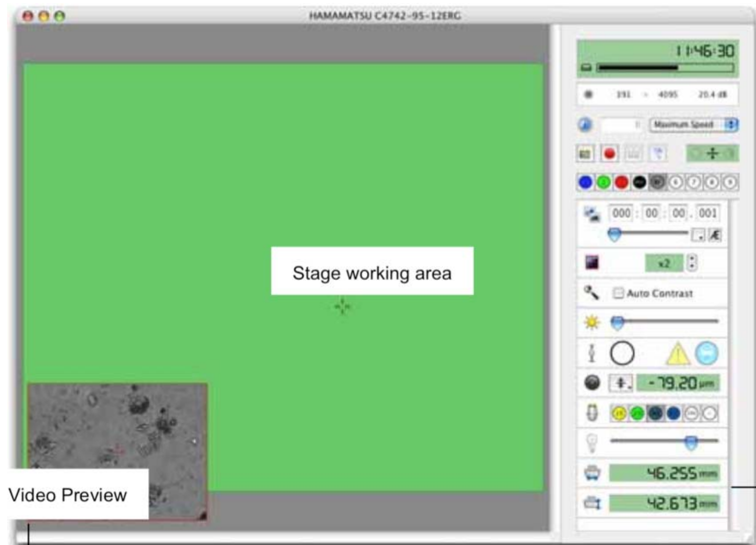
1. focus on the sample
2. click on the focus control button (Fig 4-13)
3. choose <Set Zero> to set current position as 0µm
4. find the top of the sample
5. choose <Set Top To…> and enter appropriate relative value, e.g. +5µm
6. find the bottom of the sample
7. choose <Set Bottom To…> and enter appropriate relative value, e.g. -5µm

3.2.2. Acquisition protocol setup (Fig 4-6)
Remember to balance laser power vs. bleaching, exposure time vs. dynamics; set gain accordingly, avoiding constant use of 100% gain.



1. Select the channel(s) for acquisition, e.g. multicolor
2. For Z-stack acquisition, choose one:
   - Capture using Z spacing: requires to know distance between top and bottom
   - Capture this many slices
3. Change XY: For multiple XY positions, select the method to use, default is 'Leica XY stage'
4. Setup the duration of capture; note that the frequency has to be set in the device controls
5. Specify the order for Channels and Z stacks
6. Shutter management – "balanced" usually works well
7. Save acquisition settings using a descriptive name ('save as – export' to Desktop) for future use with "Restore"

3.2.3 Multi-point acquisition



1. Make sure that **stage calibration** was done **before** any stage inserts were put in place, 'Stage : Calibrate'
2. **Clear all points** if needed (program remembers last set of points)
3. Find an XY(Z) position of interest using Video Preview, 'Stage : **Add A Point**'
   [XY points can't be modified, but have to be removed and re-added; to remove a point, select a region of interest around point, then do 'Stage : Clear Selected Point']
4. **Keep track** of point placement by sketching a map on a piece of paper
5. When all points are set, **recheck focus** by one of two methods:
   – All points sequentially: 'Stage : Review Points'; for each point, adjust focus, click 'next'
   – Points individually: 'Stage : Go to next point', adjust focus, click 'update point', repeat
6. Before starting the acquisition, quit and relaunch Volocity to **save points**, or save under a descriptive name using 'Stage : Save Points'

4 - Shutting down SD2

1. While logged on Volocity, close the **confocal fluorescence shutter** (Fig 4-16)
2. Log out of **Volocity** 3DM acquisition by closing window
3. Switch **light path** to eyepiece (Fig 3-1)
4. Turn off **lasers** (Fig 1-2), *Exfo* light source (Fig 1-16)
5. Log out of the PC, turn off the **computer** using Windows if finished using
6. Lower **objectives**, put 10x objective into position, remove stage insert
7. Turn off **SD2** - lower button on *APC* UPS (Fig 1-12)
8. **Clean** objectives by wiping off excess oil
9. Remove **stage insert**, shut down **CO_2** chamber and tank if used
10. Carefully replace the **dust cover** on the microscope
11. Note time of microscope use, comments in paper **log book**

5 - Saving data on the Server

1. Make sure to **exit Volocity** before moving any files.
2. **Connect** to the server (start menu, run: \\10.1.0.1, or create network place or shortcut), open Finder window for your folder on the SAN
3. Locate your library on the PC and drag it into your **SAN folder**
4. **Delete** the library from its original location on the hard disk as soon as possible; very large projects will have to be removed immediately after backing up. **No long term storage** will be allowed on the computer.

Note:
NEVER ACQUIRE DATA DIRECTLY ONTO THE SERVER, i.e. over the network, but acquire on the PC and then move library.
NEVER OPEN A VOLOCITY LIBRARY OVER THE NETWORK, but move it (or a copy) off the server to the computer you work on, and then open. The library could otherwise be irretrievably damaged.